The influence of starch, peptone, and sugars on the toxicity of various nitrates to Monilia sitophila (Mont.) Sacc.

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Introduction

Winogradsky and Omeliansky (7) found that the addition of 05 per cent of glucose or asparagin to nitrite-media hindered the development of the nitrate-bacteria. Peptone at a somewhat greater concentration was also detrimental. These substances are widely used in the preparation of culture media, but have been given little attention by investigators of the problems of toxicity.

Fluri (2) has reported that the salts of aluminum render the protoplasm of *Spirogyra* and other water plants permeable and are, therefore, injurious to these plants. He found, however, that if glucose, glycerin, or isodulcitol are mixed with the aluminum salt it loses its power of rendering the protoplasm permeable. Thus the toxicity of aluminum salts to these plants depends upon whether or not glucose, glycerin, or isodulcitol are present in the medium. Quite recently Schreiner and Skinner (4) have reported that the toxicity of the organic poison cumarin is counteracted by phosphates, thus indicating a relation between the organic and the inorganic part of the medium.

In view of these results it has seemed worth while to investigate the influence of organic substances on the toxicity of inorganic salts. It has been my object to determine the toxicity of different salts in the presence of certain inorganic substances that are much used in the preparation of culture media.

Material and methods

I have used the fungus *Monilia sitophila* in all of my experiments and have found it well suited to my purpose. Some of the things that recommend it are as follows: (1) It is a rapid grower. On a favorable medium at room temperature it will produce spores

in fifteen hours. (2) It is able to use as a partial source of its food supply a rather large number of organic compounds. Went (6) has found that it grows well on media containing any one of the following substances: maltose, trehalose, raffinose, saccharose, cellulose, starch, fats, and proteids. This makes it especially suitable for experiments in which the organic part of the medium is to be varied.

All of my cultures were grown in Petri dishes that were previously immersed for at least ten hours in cleaning solution made according to Duggar's method (1). In the preparation of media and for rinsing glassware redistilled water was used.

In the tables that follow, the zero sign indicates that none of the spores on the medium in question had germinated, the minus sign indicates that germination and microscopic growth had taken place, while the plus sign indicates that growth was visible to the naked eye. All cultures were incubated at room temperature (about 22° C.).

In order to determine whether or not the toxicity of various salts to *Monilia sitophila* is influenced by sugars, starch, or peptone, each of these organic substances was used separately in testing the toxicity of the inorganic salts. I have designated the highest concentration of a salt that would permit germination of the spores in a given medium, as the limit concentration for that medium. A number of preliminary experiments were made to determine the approximate value of the limit concentration for each salt in each medium used. The results obtained in a final set of experiments are shown in the tables given below.

EXPERIMENTAL

Monilia will produce a considerable growth of mycelium and will ripen spores on a medium made by adding 5 grams of cornstarch to 95 cubic centimeters of redistilled water. The results obtained in a series of experiments in which different inorganic salts were added to this medium are shown in TABLE 1. The concentrations varied from 1.33 molar for potassium nitrate to .000004 molar for zinc nitrate.

The table shows at a glance the increasing toxicity of the nitrates used, in the order in which they are arranged in the table

TABLE I

THE TOXICITY OF INORGANIC SALTS TO MONILIA SITOPHILA GROWN ON STARCH MEDIA

	Medium	Growth after 11 days
1.33	molar solution of potassium nitrate + 5% starch........	−
1.06	" " " " " + " "	−
.80	" " " " " + " "	+
.53	" " " " " + " "	+
.26	" " " " " + " "	+
.13	" " " " " + " "	+
.667	" " " calcium " + " "	−
.533	" " " " " + " "	+
.400	" " " " " + " "	+
.267	" " " " " + " "	+
.133	" " " " " + " "	+
.067	" " " " " + " "	+
.034	" " " " " + " "	+
.667	" " " sodium " + " "	o
.533	" " " " " + " "	o
.400	" " " " " + " "	−
.267	" " " " " + " "	+
.133	" " " " " + " "	+
.067	" " " " " + " "	+
.034	" " " " " + " "	+
.187	" " " barium " + " "	o
.156	" " " " " + " "	−
.125	" " " " " + " "	−
.094	" " " " " + " "	−
.062	" " " " " + " "	+
.031	" " " " " + " "	+
.007	" " " " " + " "	+
.500	" " " urea " + " "	o
.250	" " " " " + " "	o
.125	" " " " " + " "	o
.063	" " " " " + " "	o
.050	" " " " " + " "	−
.025	" " " " " + " "	+
.013	" " " " " + " "	+
.006	" " " " " + " "	+
.050	" " " ammonium " + " "	o
.038	" " " " " + " "	−
.025	" " " " " + " "	+
.013	" " " " " + " "	+
.006	" " " " " + " "	+
.0075	" " " aluminum " + " "	o
.0063	" " " " " + " "	o
.0050	" " " " " + " "	o
.0038	" " " " " + " "	o
.0025	" " " " " + " "	o
.0013	" " " " " + " "	−

.0006	molar solution of aluminum nitrate	+ 5% starch........	+
.00133	" " " ferric "	+ " "	o
.00067	" " " " "	+ " "	–
.00053	" " " " "	+ " "	o
.00040	" " " " "	+ " "	–
.00027	" " " " "	+ " "	+
.00013	" " " " "	+ " "	+
.00007	" " " " "	+ " "	+
.00133	" " " silver "	+ " "	o
.00067	" " " " "	+ " "	o
.00053	" " " " "	+ " "	o
.00040	" " " " "	+ " "	o
.00027	" " " " "	+ " "	o
.00013	" " " " "	+ " "	–
.00007	" " " " "	+ " "	+
.000338	" " " zinc "	+ " "	o
.000254	" " " " "	+ " "	o
.000169	" " " " "	+ " "	o
.000082	" " " " "	+ " "	o
.000043	" " " " "	+ " "	o
.000034	" " " " "	+ " "	o
.000025	" " " " "	+ " "	–
.000017	" " " " "	+ " "	+
.000008	" " " " "	+ " "	+
.000004	" " " " "	+ " "	+
.5000	" " " ammonium tartrate	+ " "	o
.2500	" " " " "	+ " "	o
.1250	" " " " "	+ " "	o
.0625	" " " " "	+ " "	o
.0500	" " " " "	+ " "	o
.0250	" " " " "	+ " "	–
.0125	" " " " "	+ " "	+
.0063	" " " " "	+ " "	+
	redistilled water	+ " "	+

from potassium to zinc. *Monilia* made sufficient growth to be easily visible to the naked eye in the starch medium containing potassium nitrate at a concentration of 0.8 molar or less, while at a concentration of 1.33 molar the spores germinated and produced microscopic growth. In a starch medium containing calcium nitrate at a concentration of 0.667 molar, the spores germinated but produced such a small amount of mycelium that it could be observed only with the aid of the microscope. It is interesting to note that after three days no germination had taken place in this medium. This shows that in such concentrations growth is very slow. In all media containing calcium nitrate at a concentration of 1.33 molar or less, there was abundant growth.

When sodium nitrate at a concentration of 0.533 molar was combined with the starch medium no germination occurred. When the concentration was 0.400 molar a small amount of growth was obtained. In still lower concentrations enough mycelium was produced to be easily visible to the naked eye. It was found that growth was much retarded in media containing the higher concentrations of sodium nitrate. This shows that near the limit concentration the rate of growth of *Monilia* decreases as the amount of sodium nitrate is increased.

Barium nitrate in the starch medium at a concentration of 0.187 molar inhibits the germination of the spores. At a concentration between 0.156 molar and 0.094 molar, the spores germinate but produce such a small amount of mycelium that it is not visible except under the microscope. At a concentration of 0.062 molar or less, good growth was obtained.

When the concentration of urea nitrate in the starch medium was 0.125 molar, the spores were unable to germinate. When the concentration was reduced to 0.063 molar, the spores germinated but produced such a small amount of mycelium that it was not visible to the naked eye. Good growth was obtained when the concentration of urea nitrate was 0.05 molar or less.

In starch media containing ammonium nitrate at a concentration of 0.05 molar none of the spores germinated, but when the concentration was 0.038 molar, they germinated and produced microscopic growth. When the concentration was reduced to 0.025 molar or less, abundant growth occurred.

Aluminum nitrate in starch media at a concentration of 0.0025 molar inhibits the germination of the spores. At a concentration of 0.0013 molar the spores germinate and produce microscopic mycelia, while at concentrations of 0.0006 molar or less, good growth is obtained.

Ferric nitrate in starch media is quite toxic to the spores of *Monilia*. In concentrations of 0.00027 molar or less, good growth was obtained, but in more concentrated media little or no growth occurred.

Silver nitrate is even more toxic than ferric nitrate; when its concentration was 0.00027 molar, the spores germinated, but produced only microscopic mycelia. In more concentrated media no germination took place.

Zinc nitrate in starch media is far more toxic than any of the other nitrates used; at a concentration of 0.000034 molar, it inhibited the germination of spores. In a concentration of 0.000025 molar, the spores germinated and produced microscopic mycelia, but when the zinc nitrate was used at a concentration of 0.000017 molar or less, abundant growth was obtained.

Ammonium tartrate is the only organic salt that was tried in these experiments. In starch media it is slightly more toxic than ammonium nitrate. In a concentration of 0.025 molar, the spores germinated but produced only microscopic mycelia, while in a like medium containing the same concentration of ammonium nitrate abundant growth was obtained.

As shown by TABLE I zinc nitrate is the most toxic substance used in starch media. If its limit concentration be taken as one then the limit concentrations of the other nitrates in the same medium may be expressed, by comparing equimolecular concentrations, approximately by the following numbers: silver nitrate, 5; ferric nitrate, 26; aluminum nitrate, 52; ammonium nitrate, 1,520; urea nitrate, 1,600; calcium nitrate, 16,560; and potassium nitrate, 53,200.

To show at a glance the relative toxic values of the various substances used, they are given in the order of their toxicity in TABLE II. That toxicity does not seem to be related to the valence of the kation is also shown by this table.

TABLE II

THE RELATIVE TOXICITY OF VARIOUS SALTS IN STARCH MEDIA

Valence of kation	Toxic concentration		Salt
Monovalent	1.33	molar	potassium nitrate
Divalent	.464	"	calcium "
Monovalent	.400	"	sodium "
Divalent	.156	"	barium "
Monovalent	.040	"	urea "
Monovalent	.038	"	ammonium "
Monovalent	.025	"	ammonium tartrate
Trivalent	.0013	"	aluminum nitrate
Trivalent	.00067	"	ferric "
Monovalent	.00013	"	silver "
Divalent	.000025	"	zinc "

The table shows, in the case of each salt, the greatest concentration at which growth was obtained when the salt was used

in starch media. A comparison of equimolecular concentrations shows that of all the nitrates used, potassium nitrate is the least toxic and zinc nitrate is the most toxic to *Monilia* when it is grown in a starch medium. Beginning with the least toxic, the order of toxicity of the nitrates in starch media is as follows: potassium nitrate, calcium nitrate, sodium nitrate, barium nitrate, urea nitrate, ammonium nitrate, aluminum nitrate, ferric nitrate, silver nitrate, and zinc nitrate. Having thus determined the degree of concentration at which the different nitrates are toxic to *Monilia sitophila* in starch media, experiments were made in which the organic part of the medium was varied for the purpose of determining whether or not the toxicity of these salts can be modified by the presence of one or another of the organic substances commonly used in making media. The organic substances tried are peptone, glucose, fructose, and galactose. The results obtained in these experiments are shown in TABLES III to VIII.

As shown by TABLE III, barium nitrate in peptone media at a concentration of 0.133 molar inhibits the germination of the spores of *Monilia.* In a starch medium containing barium nitrate at a concentration of 0.156 molar, the spores germinate and produce a small amount of mycelium. This shows that barium nitrate is more toxic in peptone media than in starch media. No concentration of barium nitrate shown in TABLE III was of sufficient strength to inhibit germination and growth in the presence of glucose. There was, however, a very small amount of mycelium in the media containing the barium nitrate at a concentration of 0.167 molar. This indicates that the toxic dose in glucose media is near the concentration 0.167 molar. The toxicity of barium nitrate in starch media and in glucose media is approximately the same. Its toxicity in peptone media is much greater than in glucose media or in starch media. In fructose media its toxicity is approximately the same as in starch and glucose media but is much less than in peptone media. At a concentration of 0.1 molar, barium nitrate in the presence of galactose inhibits the germination of spores. Its toxicity in galactose is approximately the same as in peptone, but is much greater than in starch, glucose, or fructose.

At a concentration of 0.033 molar, aluminum nitrate in peptone

TABLE III

THE TOXICITY OF BARIUM NITRATE IN PEPTONE, GLUCOSE, FRUCTOSE, AND GALACTOSE MEDIA

Medium	Growth after 11 days
.167 molar solution of barium nitrate + 5% peptone	o
.133 " " " " " + " "	o
.067 " " " " " + " "	−
.033 " " " " " + " "	+
.017 " " " " " + " "	+
.007 " " " " " + " "	+
.167 " " " " " + " glucose	−
.133 " " " " " + " "	−
.100 " " " " " + " "	−
.067 " " " " " + " "	−
.033 " " " " " + " "	−
.017 " " " " " + " "	+
.007 " " " " " + " "	+
.005 " " " " " + " "	+
.004 " " " " " + " "	+
.167 " " " " " + " fructose	−
.133 " " " " " + " "	−
.100 " " " " " + " "	−
.067 " " " " " + " "	−
.033 " " " " " + " "	−
.017 " " " " " + " "	−
.007 " " " " " + " "	+
.005 " " " " " + " "	+
.004 " " " " " + " "	+
.167 " " " " " + " "	o
.133 " " " " " + " "	o
.100 " " " " " + " "	o
.067 " " " " " + " "	−
.033 " " " " " + " "	−
.017 " " " " " + " "	+
.007 " " " " " + " "	+
.005 " " " " " + " "	+
.004 " " " " " + " "	+
distilled water + " peptone	+
" " + " glucose	+
" " + " fructose	+
" " + " galactose	+

media inhibits the germination of the spores; in a concentration of 0.017 molar, the spores germinate and produce microscopic mycelia. Aluminum nitrate in starch media is more than ten times as toxic as in peptone media. Its toxicity in glucose media is approximately the same as in starch media. It is much less

TABLE IV

THE TOXICITY OF ALUMINUM NITRATE IN PEPTONE, GLUCOSE, FRUCTOSE, AND GALACTOSE MEDIA

Medium	Growth after 11 days
.167 molar solution of aluminum nitrate + 5% peptone	o
.133 " " " " " + " "	o
.100 " " " " " + " "	o
.067 " " " " " + " "	o
.033 " " " " " + " "	o
.017 " " " " " + " "	–
.007 " " " " " + " "	+
.004 " " " " " + " "	+
.167 " " " " " + " glucose	o
.133 " " " " " + " "	o
.100 " " " " " + " "	o
.067 " " " " " + " "	o
.033 " " " " " + " "	o
.017 " " " " " + " "	o
.006 " " " " " + " "	o
.003 " " " " " + " "	o
.0007 " " " " " + " "	+
.167 " " " " " + " fructose	o
.133 " " " " " + " "	o
.100 " " " " " + " "	o
.067 " " " " " + " "	o
.033 " " " " " + " "	o
.017 " " " " " + " "	o
.007 " " " " " + " "	o
.002 " " " " " + " "	o
.001 " " " " " + " "	o
.0007 " " " " " + " "	–
.167 " " " " " + " galactose	o
.133 " " " " " + " "	o
.100 " " " " " + " "	o
.067 " " " " " + " "	o
.033 " " " " " + " "	o
.017 " " " " " + " "	o
.007 " " " " " + " "	o
.004 " " " " " + " "	o
.003 " " " " " + " "	o
.001 " " " " " + " "	–
.0007 " " " " " + " "	+
distilled water + " peptone	+
" " + " glucose	+
" " + " fructose	+
" " + " galactose	+

toxic in peptone media than in glucose or starch media. It is less toxic in starch media and in peptone media than in glucose media and in fructose media. In galactose it is less toxic than in fructose. Barium nitrate, on the other hand, is much more toxic in galactose media than in fructose media.

TABLE V

THE TOXICITY OF FERRIC NITRATE IN PEPTONE, GLUCOSE, FRUCTOSE, AND GALACTOSE MEDIA

Medium									Growth after 11 days
.0067	molar	solution	of	ferric	nitrate	+	5%	peptone	+
.0053	“	“	“	“	“	+	“	“	+
.0040	“	“	“	“	“	+	“	“	+
.0027	“	“	“	“	“	+	“	“	+
.0013	“	“	“	“	“	+	“	“	+
.0007	“	“	“	“	“	+	“	“	+
.0004	“	“	“	“	“	+	“	“	+
.0003	“	“	“	“	“	+	“	“	+
.0001	“	“	“	“	“	+	“	“	+
.0067	“	“	“	“	“	+	“	glucose	o
.0053	“	“	“	“	“	+	“	“	o
.0040	“	“	“	“	“	+	“	“	o
.0027	“	“	“	“	“	+	“	“	o
.0013	“	“	“	“	“	+	“	“	o
.0007	“	“	“	“	“	+	“	“	o
.0005	“	“	“	“	“	+	“	“	–
.0004	“	“	“	“	“	+	“	“	+
.0003	“	“	“	“	“	+	“	“	+
.0001	“	“	“	“	“	+	“	“	+
.0067	“	“	“	“	“	+	“	fructose	o
.0007	“	“	“	“	“	+	“	“	–
.0001	“	“	“	“	“	+	“	“	+
.0067	“	“	“	“	“	+	“	galactose	o
.0053	“	“	“	“	“	+	“	“	o
.0040	“	“	“	“	“	+	“	“	o
.0027	“	“	“	“	“	+	“	“	o
.0013	“	“	“	“	“	+	“	“	o
.0007	“	“	“	“	“	+	“	“	o
.0005	“	“	“	“	“	+	“	“	o
.0004	“	“	“	“	“	+	“	“	–
.0003	“	“	“	“	“	+	“	“	+
.0001	“	“	“	“	“	+	“	“	+
				distilled	water	+	“	peptone	+
				“	“	+	“	glucose	+
				“	“	+	“	fructose	+
				“	“	+	“	galactose	+

As shown by TABLE V, ferric nitrate at a concentration of 0.0067 molar, does not hinder the growth of *Monilia* in peptone media. In glucose media it inhibits the germination of the spores, when present at a concentration of 0.0007 molar. The small amount of mycelium obtained in fructose media containing ferric nitrate at a concentration of 0.0007 molar indicates that this is near its limit concentration. It is more toxic in galactose than in fructose media,—but is much less toxic in peptone than in any of the other media used.

TABLE VI

THE TOXICITY OF UREA NITRATE AND AMMONIUM NITRATE IN PEPTONE MEDIA

Medium		Growth after 11 days
.200 molar solution of urea nitrate	+ 5% peptone........	−
.133 " " " " "	+ " "	+
.067 " " " " "	+ " "	+
.033 " " " " "	+ " "	+
.027 " " " " "	+ " "	+
.020 " " " " "	+ " "	+
.013 " " " " "	+ " "	+
.007 " " " " "	+ " "	+
.133 " " " ammonium tartrate	+ " "	o
.067 " " " " "	+ " "	o
.060 " " " " "	+ " "	o
.053 " " " " "	+ " "	o
.047 " " " " "	+ " "	o
.040 " " " " "	+ " "	o
.033 " " " " "	+ " "	o
.027 " " " " "	+ " "	−
.020 " " " " "	+ " "	−
.013 " " " " "	+ " "	−
.007 " " " " "	+ " "	+
distilled water	+ " "	+

The small amount of growth obtained in peptone media containing urea nitrate at a concentration of 0.2 molar indicates that this is approximately its limit concentration. In peptone media containing ammonium tartrate at a concentration of 0.027 molar, only microscopic mycelia were obtained. The toxicity of urea nitrate in starch media is four times as great as its toxicity in peptone media. The toxicity of ammonium tartrate, on the other hand, is approximately the same in starch as in peptone media.

TABLE VII

THE INFLUENCE OF PEPTONE ON THE TOXICITY OF FERRIC NITRATE IN STARCH MEDIA

Medium	Growth after 11 days
.0067 molar solution of ferric nitrate + 5% starch	o
.0067 " " " " " + " " + 1% peptone	o
.0067 " " " " " + " " + 2% "	−
.0067 " " " " " + " " + 3% "	−
.0067 " " " " " + " " + 4% "	+
.0067 " " " " " + " " + 5% "	+
.0067 " " " " " + " " + 6% "	+
.0067 " " " " " + " " + 7% "	+
.0067 " " " " " + " " + 8% "	+
.0067 " " " " " + " " + 9% "	+

TABLE VII shows the degree to which peptone counteracts the toxicity of ferric nitrate in starch media. When only 1 per cent of peptone was added to this medium none of the spores germinated; when 2 per cent of peptone was added the spores germinated and produced microscopic mycelia, but when 4 per cent of peptone was added vigorous growth was obtained.

TABLE VIII

THE TOXICITY OF VARIOUS SALTS IN SOME COMMON MEDIA

Toxic concentration	Salt	Medium
.156 molar	barium nitrate	starch
.067 "	" "	peptone
.167 "	" "	glucose
.167 "	" "	fructose
.067 "	" "	galactose
.0013 "	aluminum "	starch
.0170 "	" "	peptone
.0070 "	" "	glucose
.0007 "	" "	fructose
.0013 "	" "	galactose
.00067 "	ferric "	starch
.00670 "	" "	peptone
.00050 "	" "	glucose
.00070 "	" "	fructose
.00040 "	" "	galactose
.05 "	urea "	starch
.20 "	" "	peptone
.025 "	ammonium tartrate	starch
.027 "	" "	peptone

As shown by the above table, barium nitrate is more toxic in peptone and galactose than in the other media. Aluminum nitrate

and ferric nitrate, on the other hand, are far less toxic in peptone than in the other substances used. Urea nitrate is more toxic in starch media than in peptone media, while the toxicity of ammonium tartrate is the same in the two media.

Discussion

My observations show beyond question that the concentration at which various inorganic salts are toxic to *Monilia sitophila* depends on the kind of organic substances contained in the media to which those salts are added. The same substance at a given concentration may be highly toxic in one medium but quite harmless in another. The concentration of ferric nitrate that inhibits the growth of *Monilia* in starch media has little or no effect upon its growth in peptone media. Lipman (3), using a peptone medium, found that calcium chloride is more toxic to *Bacillus subtilis* than is potassium chloride, sodium chloride, or magnesium chloride. My experiments suggest that he might have obtained a different result by adding these chlorides to a medium containing starch or sugar instead of peptone. Ssadikow (5) has tested the toxicity of strychnin salts to *Bacillus subtilis*, using as his media, bouillon, nutrient agar, and nutrient gelatin. He found this organism able to withstand a surprisingly high concentration of these salts. A very different result might have been obtained if the strychnin salts had been added to media lacking in peptone.

We have in such experiments, it seems to me, a means of attacking the whole question as to the nature of toxicity and toxic effects from an important and rather neglected standpoint and I hope to extend this work to a study of still other combinations of toxic and non-toxic substances as regards their effects on various living organisms. It is also obvious that such data are necessary to a proper understanding of the effects of various food substances, drugs, etc., both from the standpoint of the significance of the medium with which the chemical is combined when offered as a food and from the standpoint of its relation to the organic reserve and other substances which it meets in the cell.

SUMMARY

1. The degree of toxicity of barium nitrate, aluminum nitrate, ferric nitrate, and urea nitrate to *Monilia sitophila* depends on the organic substance contained in the media in which these salts are offered.

2. Barium nitrate is more toxic in peptone media than in starch media, while aluminum nitrate and ferric nitrate are more toxic in starch media than in peptone media.

3. Barium nitrate has practically the same toxicity in starch media that it has in either glucose or fructose media. Its toxicity in peptone media is the same as its toxicity in galactose media.

4. The toxicity of aluminum nitrate in galactose media is the same as its toxicity in starch media. Its toxicity in fructose is much greater than in peptone or glucose.

5. The toxicity of ferric nitrate is approximately the same in starch as in glucose, fructose, or galactose. Its toxicity in peptone, however, is much less than in any of the other substances used.

6. Urea nitrate is four times more toxic in starch media than in peptone media.

7. The toxicity of ammonium tartrate is practically the same in starch as in peptone media.

8. If the limit concentration of zinc nitrate in starch media be taken as 1, then the limit concentration of the other nitrates in the same kind of media may be expressed, by comparing equimolecular concentrations, approximately by the following numbers: silver nitrate, 5; ferric nitrate, 26; aluminum nitrate, 52; ammonium nitrate, 1,520; urea nitrate, 1,600; calcium nitrate, 16,560; and potassium nitrate, 53,200.

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